Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time in Japanese wheat cultivars

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The genotypes of photoperiod response genes *Ppd-B1* and *Ppd-D1* in Japanese wheat cultivars were determined by a PCR-based method, and heading times were compared among genotypes. Most of the Japanese wheat cultivars, except those from the Hokkaido region, carried the photoperiod-insensitive allele *Ppd-D1a*, and heading was accelerated 10.3 days compared with the *Ppd-D1b* genotype. Early cultivars with *Ppd-D1a* may have been selected to avoid damage from preharvest rain. In the Hokkaido region, *Ppd-D1a* frequency was lower and heading date was late regardless of *Ppd-D1* genotype, suggesting another genetic mechanism for late heading in Hokkaido cultivars. In this study, only 11 cultivars proved to carry *Ppd-B1a*, and all of them carried another photoperiod-insensitive allele, *Ppd-D1a*. The *Ppd-B1a*/*Ppd-D1a* genotype headed 6.7 days earlier than the *Ppd-B1b*/*Ppd-D1a* genotype, indicating a significant effect of *Ppd-B1a* in the genetic background with *Ppd-D1a*. Early-maturity breeding in Japan is believed to be accelerated by the introduction of the *Ppd-B1a* allele into medium-heading cultivars carrying *Ppd-D1a*. Pedigree analysis showed that *Ppd-B1a* in three extra-early commercial cultivars was inherited from 'Shiroboro 21' by early-heading Chugoku lines bred at the Chugoku Agriculture Experimental Station.

Key Words: wheat, photoperiod response gene, *Ppd-B1*, *Ppd-D1*, heading date, Japanese wheat cultivars.

Introduction

Wheat (*Triticum aestivum* L.) should be harvested before the rainy season in Japan, except in the Hokkaido area, to avoid damage such as preharvest sprouting and Fusarium head blight. Therefore, early heading is one of the most important traits in wheat breeding. However, shorter growth periods generally result in lower grain yields, and early-heading wheat cultivars with early apical development and stem elongation are prone to frost injury (Hukumoto and Takahashi 1950, Taya 1993). Thus, heading characteristics must be adjusted for the stable production of wheat in each area.

Heading time of wheat is a complex character controlled by narrow-sense earliness (also termed earliness *per se*) and is modified by vernalization responses and photoperiod responses (Kato and Yamashita 1991, Yasuda and Shimoyama 1965). It was also reported that photoperiod response is the major determinant of earliness in autumn-sown wheat in central and southwestern Japan (Tanio *et al.* 2006, Yasuda and Shimoyama 1965, Yoshida *et al.* 1983), and that heading time is independent of the *Vrn* genotype controlling vernalization response (Fujita *et al.* 1995, Kato and Yamashita 1991, Tanio *et al.* 2005).

Photoperiod response is controlled mainly by three major genes, namely, *Ppd-D1* (previously designated *Ppd1*), *Ppd-B1* (*Ppd2*) and *Ppd-A1* (*Ppd3*), located on homoeologous group two chromosomes (Scarth and Law 1983, 1984, Welsh *et al.* 1973). The barley homoeologue *Ppd-H1* was identified as a member of the pseudo-response regulator (*PRR*) gene family (Turner *et al.* 2005). The orthologous *PRR* genes of the A, B and D genomes have been isolated from wheat BAC (bacterial artificial chromosome) libraries, and sequence analyses revealed that the photoperiod-insensitive *Ppd-D1a* allele is associated with a 2,089-bp deletion upstream of the coding region (Beales *et al.* 2007). Recent data show that the photoperiod-insensitive *Ppd-A1a* and *Ppd-B1a* alleles are associated with a 1085-bp deletion and a 308-bp insertion, respectively (Nishida *et al.* submitted, GenBank sequence accessions are AB646973 and AB646974), both of which share the common region with a deletion of *Ppd-D1a*.

Based on the sequence polymorphism among *Ppd-D1* alleles reported by Beales *et al.* (2007), Yang *et al.* (2009) determined the *Ppd-D1* genotype of Chinese wheat landraces and indicated that *Ppd-D1a* allele frequency varies among different areas, even within China. The *Ppd-D1a* allele was

not found in northern China but was found frequently in southeastern China. Most of the European wheat cultivars with photoperiod insensitivity probably carry *Ppd-D1a* derived from 'Akakomugi' (Worland 1996). This assumption is supported by Guo *et al.* (2010), who showed that most Italian wheat cultivars carry *Ppd-D1a*. Tanio *et al.* (2005) analyzed the *Ppd-1* genotype of Japanese wheat cultivars by conventional segregation analysis and reported the following results. The very late-heading cultivar 'Haruhikari' does not carry any major photoperiod-insensitive alleles. Medium-to late-heading cultivars such as 'Norin 61' and 'Saitama 27' carry a single allele for photoperiod insensitivity. Extremely early-heading cultivars carry two alleles for photoperiod insensitivity. Lately, it was revealed that the former single allele is *Ppd-D1a* and the latter two alleles are *Ppd-B1a* and *Ppd-D1a* by Nishida *et al.* (submitted). However, only eight cultivars were analyzed because the conventional segregation procedure is labor intensive. On the contrary, genotyping based on the detection of sequence differences allows for the analysis of many wheat cultivars and a comprehensive analysis of the relationship between the *Ppd-1* genotype and the heading time of Japanese wheat cultivars.

In this study, the *Ppd-B1* and *Ppd-D1* genotypes of Japanese wheat cultivars were determined by a PCR-based method to detect large deletions or insertions in the upstream region of the respective gene. Heading date in the field was compared among wheat cultivars carrying a sensitive or insensitive allele of each gene, and the effect of *Ppd-1* genotypes on heading time was successfully evaluated. In addition, the pedigree of extremely early-heading cultivars was discussed based on *Ppd-1* genotype.

Materials and Methods

A total of 260 wheat cultivars, consisting of Japanese commercial cultivars (157 cultivars), Japanese breeding lines (45 cultivars), Japanese landraces (25 cultivars) and foreign cultivars introduced for breeding (33 cultivars), were used in the present study. Geographical origins are summarized in Table 1. The cultivars were grown in a field at the NARO Institute of Crop Science (36°01′N, 140°06′E) in the Kanto region of Japan and were sown on November 4, November 10 and October 31 for the 2004/2005, 2005/2006 and 2006/2007 wheat growing seasons, respectively. Each experimental plot consisted of a single 1.0-m-long row, and the planting distance was 70 cm between rows and 8.5 cm between plants. Heading date was recorded when the tip of the first ear emerged from the flag leaf sheath in one-half of the plants for each cultivar.

For DNA extraction, all wheat genotypes were grown in a growth chamber maintained at 20°C under a natural photoperiod. Genomic DNA was extracted from 2-week-old seedlings using a modified CTAB method (Murray and Thompson 1980).

Ppd-B1 and *Ppd-D1* genotypes were determined using PCR-based methods with the primer sets designed to identi-

Table 1. Distribution of photoperiod insensitive alleles *Ppd-B1a* and *Ppd-D1a* in Japanese and foreign wheat cultivars

Area of origin	Total number of cultivars	*Ppd-B1*		*Ppd-D1*	
		Ppd-B1a	*Ppd-B1b*	*Ppd-D1a*	*Ppd-D1b*
Hokkaido (Winter wheat)	19	0	19	7	12
Hokkaido (Spring wheat)	10	0	10	1[f]	9
Tohoku, Hokuriku	47	0	47	43	4[g]
Kanto, Tokai	62	2[b]	60	62	0
Kinki, Chugoku, Shikoku	42	6[c]	36	41	1[h]
Kyushu	47	2[d]	45	47	0
Foreign cultivars [a]					
High latitude area	11	0	11	4	7
Low latitude area	22	1[e]	21	13	9
Total	260	11	249	218	42

[a] High and low latitude areas are tentatively separated by 40 degrees.
[b] Carrier of *Ppd-B1a*; 'Konosu 4' and 'Shiroboro 21'
[c] Carrier of *Ppd-B1a*; 'Chugoku 55', 'Chugoku 81', 'Chugoku 91', 'Chugoku 98', 'Chugoku 114' and 'Fukuwasekomugi'
[d] Carrier of *Ppd-B1a*; 'Sakigakekomugi' and 'Abukumawase'
[e] Carrier of *Ppd-B1a*; 'Tapdongmil'
[f] Carrier of *Ppd-D1a*; 'OS-21'
[g] Carrier of *Ppd-D1b*; 'Fultz Daruma', 'Norin 6', 'Norin 24' and 'Norin 38'
[h] Carrier of *Ppd-D1b*; 'Eshima'

Table 2. Primers used to determine the *Ppd-D1* and *Ppd-B1* genotypes

Locus	Primer name	Sequence (5′→3′)
Ppd-B1	TaPpd-B1proF1	ACACTAGGGCTGGTCGAAGA
	TaPpd-B1int1R1	CCGAGCCAGTGCAAATTAAC
Ppd-D1	TaPpd-D1_F1	ACGCCTCCCACTACACTG
	TaPpd-D1_R1	TGTTGGTTCAAACAGAGAGC
	TaPpd-D1_R2	CACTGGTGGTAGCTGAGATT

fy the deletion of 2089 bp in the upstream region of *Ppd-D1a* (Beales *et al.* 2007) or the insertion of 308 bp in the upstream region of *Ppd-B1a* (Nishida *et al.* submitted). Three primers, namely, Ppd-D1_F1, Ppd-D1_R1 and Ppd-D1_R2 (developed by Beales *et al.* 2007), were used for *Ppd-D1*, and two primers, i.e., TaPpd-B1proF1 and TaPpd-B1int1R1 (developed in this study), were used for *Ppd-B1*. The nucleotide sequence of each primer is shown in Table 2.

For the analysis of *Ppd-D1*, PCR amplification was performed in a 5-μl mixture containing 10 ng genomic DNA, 0.5 μl 10× *Ex Taq* buffer (TaKaRa, Japan; 20 mM Tris-HCl at pH 8.0, 100 mM KCl, 20 mM Mg^{2+}), 0.2 mM dNTP, 0.2 μM of each primer and 0.125 U *Ex Taq* Hot Start Version (TaKaRa). The PCR cycle was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 54°C for 1 min and 72°C for 30 sec; and a final extension step at 72°C for 2 min. For the analysis of *Ppd-B1*, PCR amplification was performed in a 5-μl mixture containing 10 ng genomic DNA, 0.5 μl 10× *Pyrobest* buffer (TaKaRa; 50 mM Tris-HCl at pH 8.2, 10 mM Mg^{2+}), 0.2 mM

dNTP, 0.2 µM of each primer and 0.125 U *Pyrobest* DNA polymerase (TaKaRa). The PCR cycle was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 64°C for 1 min, and 72°C for 30 sec and a final extension step at 72°C for 2 min. Amplification reactions were conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, USA). PCR products were electrophoresed on acrylamide gel. Electrophoresis and polymorphism detection were based on the description by Hori *et al.* (2003).

Heading date data were analyzed using statistical software (SPSS Ver. 18.0 J for Windows, SPSS Japan Inc.).

Results

Expected PCR product sizes, i.e., 288 bp from *Ppd-D1a* or 415 bp from *Ppd-D1b*, photoperiod-sensitive allele without a deletion of 2089 bp, were successfully amplified by multiplex PCR in all of the cultivars tested. For *Ppd-B1*, 1600 bp from *Ppd-B1a* or 1292 bp from *Ppd-B1b*, photoperiod-sensitive allele without an insertion of 308 bp, were successfully amplified in all of the cultivars tested. Among 260 cultivars, 218 cultivars (83.8%) proved to carry the photoperiod-insensitive allele *Ppd-D1a* (Table 1). Frequency of the *Ppd-D1a* genotype was different among geographical groups. Only seven cultivars of winter wheat carried the *Ppd-D1a* allele in Hokkaido, whereas 193 of 198 Japanese wheat cultivars (97.5%), except those from the Hokkaido region, and half of the foreign cultivars (51.5%) carried the *Ppd-D1a* allele (Table 1 and Fig. 1) . On the other hand, only 11 cultivars (4.2%) carried *Ppd-B1a* allele (Table 1). Among them, two accessions from the Kanto and Tokai regions and six accessions from the Kinki, Chugoku and Shikoku regions included an extra-early cultivar, 'Fukuwasekomugi', and five breeding lines. Two cultivars from the Kyushu region, i.e., 'Sakigakekomugi' and 'Abukumawase' and one cultivar from Korea, i.e., 'Tapdongmil', also carried *Ppd-B1a* allele (Table 1 and Fig. 2) . All of the cultivars with *Ppd-B1a* allele carried *Ppd-D1a* allele as well; the *Ppd-B1a/Ppd-D1b* genotype was not found in this study.

As summarized in Table 3, heading date of wheat cultivars differed significantly among the three seasons as well as among *Ppd-D1* genotypes (P ≤ 0.001). As shown in Fig. 3, monthly average temperatures differed among the three wheat growing seasons. Compared with average temperatures for the last 30 years, temperatures during the early growing stage of wheat were higher in the 2004/2005 season and lower in the 2005/2006 season. In contrast, a warm winter in 2006/2007 resulted in higher temperatures for the entire growing season.

Average heading date of the *Ppd-D1a* and *Ppd-D1b* genotypes were 28.2 April and 5.7 May, respectively, for the 2004/2005 season. A similar genotypic difference was observed in the other two seasons, indicating that the *Ppd-D1a* genotype headed approximately 10 days earlier than the *Ppd-D1b* genotype. The genotypic difference was not signif-
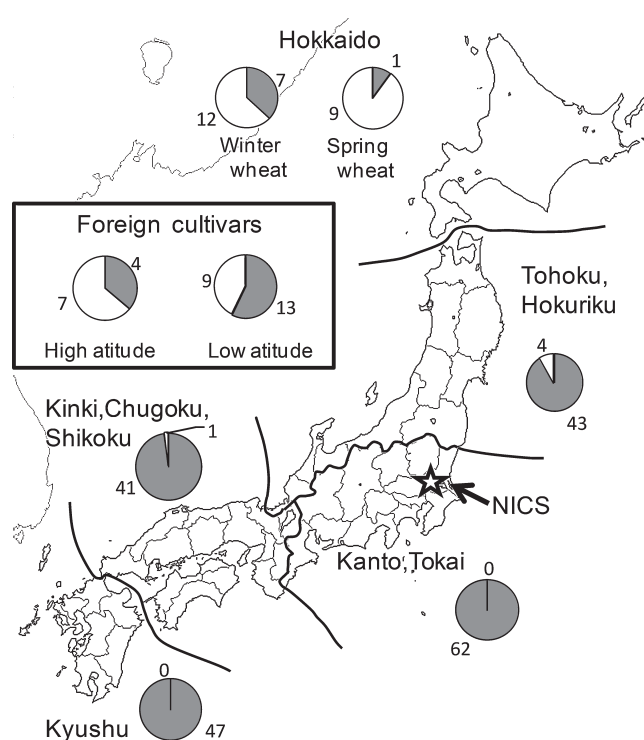

Fig. 1. Geographical distribution of *Ppd-D1a* and *Ppd-D1b*. Solid and open parts of the circular charts indicate the proportions of wheat cultivars carrying *Ppd-D1a* and *Ppd-D1b*, respectively. Number of cultivars was also indicated.

icant in wheat cultivars from the Hokkaido region, where winter wheat and spring wheat are grown in different areas. For winter wheat cultivars in this region, average heading date of the *Ppd-D1a* and *Ppd-D1b* genotypes were 7.1 May and 7.8 May, respectively, for the 2004/2005 season. In foreign cultivars from high-latitude areas, the *Ppd-D1a* genotype headed 5–6 days earlier than the *Ppd-D1b* genotype. The genotypic differences in each season were not significant. However, ANOVA results showed that the *Ppd-D1* genotype was significant for heading at the 5% level.

Heading date of the three genotypes detected in the present study, namely, *Ppd-B1a/Ppd-D1a*, *Ppd-B1b/Ppd-D1a* and *Ppd-B1b/Ppd-D1b*, are shown in Table 4. Because wheat cultivars of the *Ppd-B1a/Ppd-D1b* genotype were not detected, the effect of the *Ppd-B1* gene under the *Ppd-D1b* genetic background could not be analyzed. Therefore, heading date was compared between two genotypes, *Ppd-B1a/Ppd-D1a* and *Ppd-B1b/Ppd-D1a*, to determine the interaction between the *Ppd-B1a* and *Ppd-D1a* alleles. Heading date of *Ppd-B1a/Ppd-D1a* and *Ppd-B1b/Ppd-D1a* genotypes were 22.1 April and 28.5 April for the 2004/2005 season, respectively; similar results were obtained for the other two seasons. Heading date of photoperiod-insensitive wheat cultivars carrying *Ppd-D1a* was accelerated by 4.9–8.7 days when combined with *Ppd-B1a*. Heading time stability among the three seasons also differed depending on the *Ppd-1* genotype. Heading time differences between the 2005/2006 season
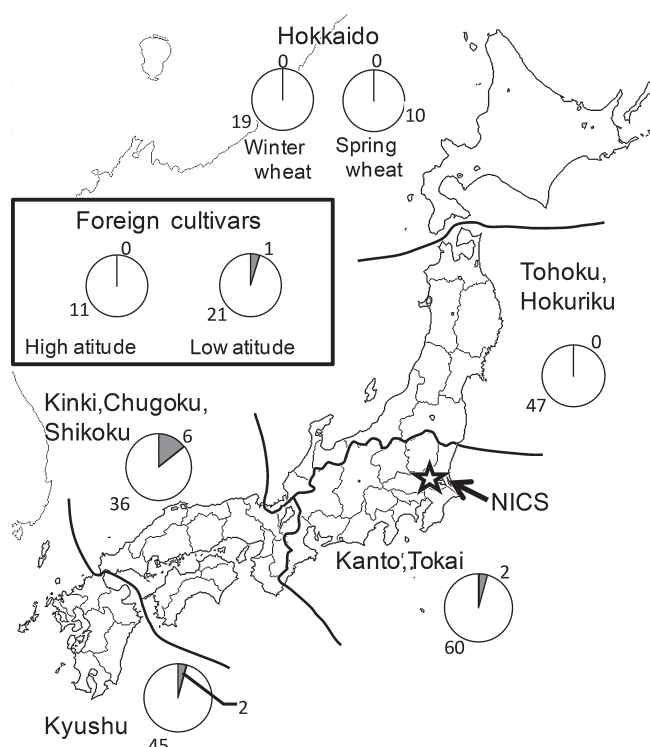
Fig. 2. Geographical distribution of *Ppd-B1a* and *Ppd-B1b*. Solid and open parts of the circular charts indicate the proportions of wheat cultivars carrying *Ppd-B1a* and *Ppd-B1b*, respectively. Number of cultivars was also indicated.

Fig. 3. Monthly mean temperature at Tsukuba. ——: Average of the last 30 years. ----: 2004/2005 growing season. ······: 2005/2006 growing season. -·-·-: 2006/2007 growing season.

The *Ppd-B1* genotypes of wheat lines in the pedigree of extremely early wheat cultivars carrying *Ppd-B1a* allele ('Sakigakekomugi', 'Fukuwasekomugi' and 'Abukumawase') are summarized in Fig. 4. Four early-heading breeding lines, 'Chugoku 55', 'Chugoku 81', 'Chugoku 91' and 'Chugoku 114', bred at the Chugoku Agriculture Experimental Station proved to carry *Ppd-B1a*. The old cultivars 'Konosu 4' and 'Shiroboro 21' also carried *Ppd-B1a*, whereas the other cultivars carried *Ppd-B1b* (Fig. 4). This result indicated that *Ppd-B1a* of extremely early wheat cultivars was inherited from 'Shiroboro 21' by early-heading Chugoku lines, although the *Ppd-1* genotype was not determined for 'Kinki 14' because seed was not available.

with a cold winter and the 2006/2007 season with a warm winter was 15.9 days in the *Ppd-B1a*/*Ppd-D1a* genotype and 12.0–12.5 days in the other *Ppd-1* genotypes (Table 5).
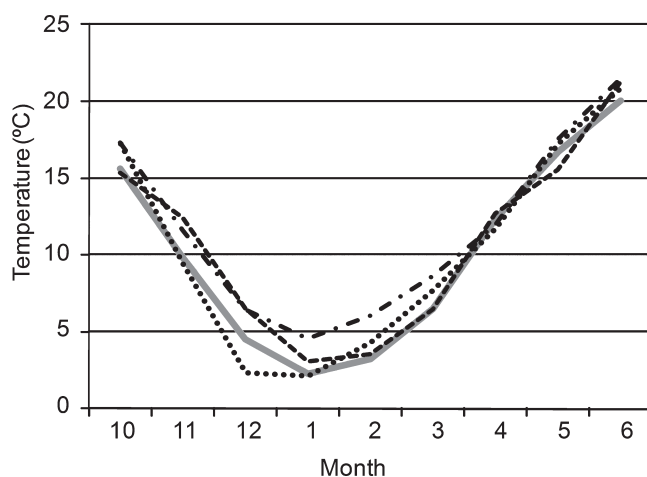
Table 3. Average of heading date of each *Ppd-D1* genotype

Growing season	*Ppd-D1* genotype	Total		Hokkaido						Tohoku-Kyushu		Foreign cultivars					
				Total		Winter wheat						Total		High latitude area [b]		Low latitude area [b]	
		n	Heading date [a]	n	Heading date	n	Heading date		n	Heading date	n	Heading date	n	Heading date	n	Heading date
2004/2005	*Ppd-D1a*	216	28.2 ± 0.3	8	36.1 ± 1.5	7	37.1 ± 1.3		192	27.8 ± 0.3	16	28.2 ± 1.2	4	32.0 ± 1.3	12	26.9 ± 1.4
	Ppd-D1b	42	35.7 ± 0.8	21	36.3 ± 0.9	12	37.8 ± 1.1		5	36.0 ± 3.3	16	34.9 ± 1.5	7	37.3 ± 2.8	9	33.0 ± 1.4
			***		ns		ns			***		***		ns		***
2005/2006	*Ppd-D1a*	216	32.1 ± 0.3	8	40.3 ± 1.9	7	40.7 ± 2.1		192	31.8 ± 0.2	16	32.6 ± 1.7	4	38.3 ± 1.0	12	30.7 ± 1.9
	Ppd-D1b	42	43.0 ± 0.9	21	44.1 ± 1.2	12	43.8 ± 1.7		5	41.8 ± 3.5	16	41.8 ± 1.5	7	43.3 ± 2.7	9	40.7 ± 1.7
			***		ns		ns			***		***		ns		***
2006/2007	*Ppd-D1a*	216	19.9 ± 0.4	8	29.6 ± 1.7	7	30.5 ± 1.6		192	19.5 ± 0.4	16	19.8 ± 1.6	4	24.0 ± 1.4	12	18.4 ± 1.9
	Ppd-D1b	42	30.5 ± 0.8	21	31.6 ± 1.0	12	32.2 ± 1.2		5	32.2 ± 3.1	16	28.4 ± 1.5	7	30.0 ± 2.7	9	27.1 ± 1.6
			***		ns		ns			***		***		ns		***
F-value of ANOVA [c]																	
Growing seoson (A)			225.2 ***		34.30 ***		24.70 ***			30.27 ***		38.66 ***		13.33 ***		27.93 ***
Ppd-D1 genotype (B)			410.1 ***		3.133 ns		1.906 ns			80.45 ***		44.84 ***		6.217 *		34.18 ***
(A)*(B)			4.775 *		0.850 ns		0.311 ns			1.300 ns		0.394 ns		0.018 ns		0.664 ns

[a] Values showed mean ± standard error. 1 = 1st April. *** and 'ns' indicate significance at 0.1% levels and no significance at 5% level, respectively, by T-test.

[b] High and low latitude areas are tentatively separated by 40 degrees.

[c] * and *** indicate significance at 5% and 0.1% level, respectively, and 'ns' not significant.
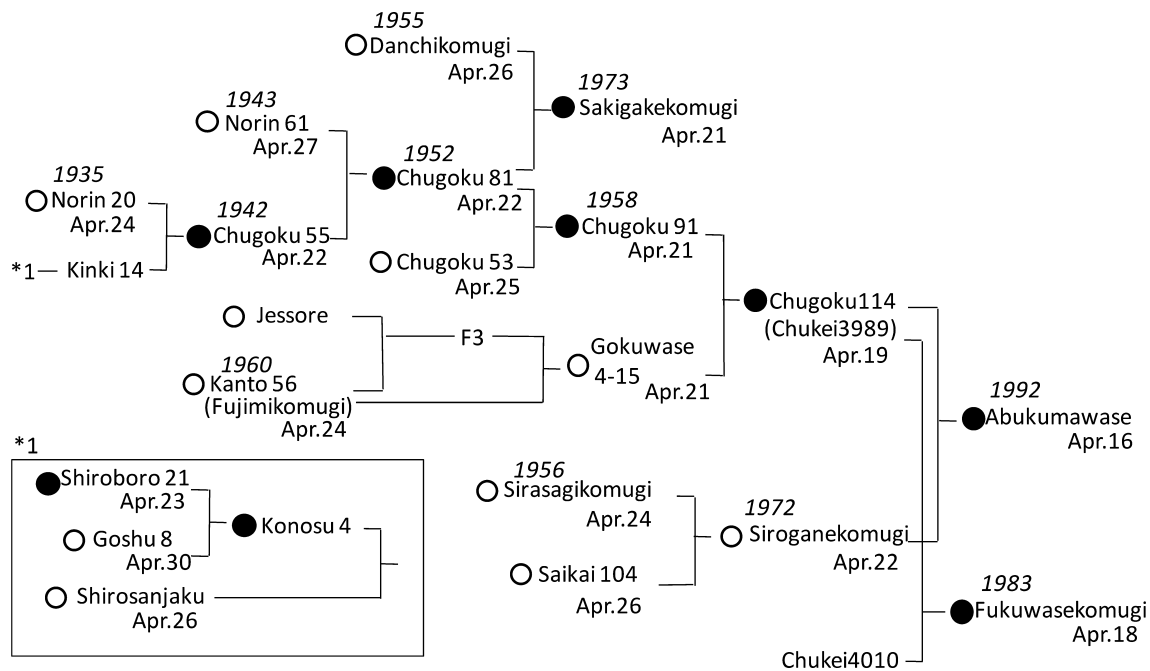
Fig. 4. *Ppd-B1* genotype and heading date of wheat cultivars on the pedigree of extra early wheat cultivars. ●: Carrier of *Ppd-B1a*. ○: Carrier of *Ppd-B1b*. *Ppd-1* genotype and heading date of 'Kinki 14' and 'Chukei 4010' and heading date of 'Jessore' and 'Konosu 4' were not tested. The average of heading date for three growing seasons is indicated by calendar date. The year of registration was shown in italics. *1 shows the pedigree of 'Kinki 14'.

Table 4. Additive effect of *Ppd-D1a* and *Ppd-B1a* for heading date

Growing season	*Ppd-1* genotype	Number of cultivars	Heading date [a]
2004/2005	*Ppd-B1a/Ppd-D1a*	10	22.1 ± 0.8[a]
	Ppd-B1b/Ppd-D1a	206	28.5 ± 0.3[b]
	Ppd-B1b/Ppd-D1b	42	35.7 ± 0.8[c]
2005/2006	*Ppd-B1a/Ppd-D1a*	10	27.5 ± 0.7[a]
	Ppd-B1b/Ppd-D1a	206	32.4 ± 0.3[b]
	Ppd-B1b/Ppd-D1b	42	43.0 ± 0.9[c]
2006/2007	*Ppd-B1a/Ppd-D1a*	10	11.6 ± 0.8[a]
	Ppd-B1b/Ppd-D1a	206	20.3 ± 0.4[b]
	Ppd-B1b/Ppd-D1b	42	30.5 ± 0.8[c]
F-value of ANOVA [b]			
	Growing seoson (A)		144.4 ***
	Ppd-1 genotype (B)		208.7 ***
	(A)*(B)		3.380 **

[a] Values showed mean ± standard error. 1 = 1st April. Values with the different letter indicate significant difference (P < 0.001) by Tukey HSD multiple range test, for each growing season.
[b] ** and *** indicate significance at 1% and 0.1% level, respectively.

Table 5. Difference of heading time between 2005/2006 (cold winter) and 2006/2007 (warm winter)

Ppd-1 genotype	Number of cultivars	Difference of heading date [a]
Ppd-B1a/Ppd-D1a	10	15.9 ± 0.5[a]
Ppd-B1b/Ppd-D1a	206	12.0 ± 0.2[b]
Ppd-B1b/Ppd-D1b	42	12.5 ± 0.4[b]
F-value of ANOVA [b]		
Ppd-1 genotype		7.173**

[a] Values showed mean ± standard error. Values with the different letter indicate significant difference (P < 0.01) by Tukey HSD multiple range test.
[b] ** indicates significance at 1% level.

Discussion

Examination of the *Ppd-D1* genotypes of 227 Japanese wheat cultivars by PCR-based analysis detected a deletion of 2089 bp in the 5′ upstream region (Beales *et al*. 2007), showing that 201 cultivars (88.4%) carried the insensitive allele *Ppd-D1a* (Table 1). In the Tohoku-Kyushu region, most of the cultivars (97.5%) carried *Ppd-D1a*. The predominance of the *Ppd-D1a* allele was also found in Chinese improved wheat cultivars (90.6%, Yang *et al*. 2009) and Pakistani spring bread wheat cultivars (98.3%, Iqbal *et al*. 2011). According to Guo *et al*. (2010), *Ppd-D1a* was carried in 11 of 12 Italian cultivars and in all 29 Mexican cultivars but was uncommon in Europe and North America except for Italy and Mexico. For such a geographical difference, Worland *et al*. (1996) explained as follows: *Ppd-D1a* accelerates flowering by 4–8 days, so that only the early genotype carrying *Ppd-D1a* is able to fill grain before the hot, dry summer season in southern Europe. Our result also indicated that *Ppd-D1a* accelerates heading by 8.2–12.7 days in the Tohoku-Kyushu cultivars (Table 3). In Japan, except in Hokkaido, the rainy season starts before the wheat harvest; thus, early cultivars with *Ppd-D1a* have been selected to avoid damage

such as preharvest sprouting and Fusarium head blight.

Ppd-D1a frequency in the Hokkaido cultivars was 27.6%, which was lower than *Ppd-D1a* frequency in the Tohoku-Kyushu region (Table 1 and Fig. 1). *Ppd-D1a* also was less frequent in wheat landraces from northern China than in wheat landraces from southern China (Yang *et al.* 2009). In addition, heading dates of the *Ppd-D1a* genotype was not significantly different from that of the *Ppd-D1b* genotype in Hokkaido cultivars, and was similar to that of *Ppd-D1b* genotype in the Tohoku-Kyushu cultivars. The lateness of the Hokkaido cultivars is an important trait for adaptation to longer winters. However, in foreign cultivars from high-latitude areas, heading date differed significantly among the *Ppd-D1* genotype (Table 3, P < 0.05). According to Foulkes *et al.* (2004) who analyzed the NILs of United Kingdom cultivars 'Merica' and 'Cappela-Desprez', differing in *Ppd-D1* genotypes, flag leaf unfolding was 12.5 days earlier in *Ppd-D1a* NILs than in *Ppd-D1b* NILs. These findings suggest that the *Ppd-D1a* genotype of the Hokkaido cultivar has another genetic mechanism for late heading. Although this mechanism is unknown, foreign wheat cultivars have been introduced from Europe and the United States for breeding with Hokkaido cultivars (Fukunaga and Inagaki 1985, Hoshino *et al.* 2001); thus, the genetic background of Hokkaido cultivars is considered different from that of wheat cultivars in other areas of Japan. Another possibility is the functional difference among the *Ppd-D1a* alleles, though *Ppd-D1a* allele of the Hokkaido cultivars has not been sequenced. Further study is required to uncover the genetic factors involved in the control of heading time in Hokkaido cultivars.

Although 'Chinese Spring' carries the photoperiod-insensitive allele *Ppd-B1* (Law *et al.* 1978, Scarth and Law 1983), a 308-bp insertion was not detected in the 5′ upstream region, and this result shows that 'Chinese Spring' carried an allele that was different from *Ppd-B1a* (Nishida *et al.* submitted). In the present study, 11 cultivars were proved to carry *Ppd-B1a* with the 308-bp insertion, as well as *Ppd-D1a*. Among them, 'Fukuwasekomugi' and 'Abukumawase' were already confirmed to have *Ppd-B1a* by conventional segregation or molecular genetics analysis (Nishida *et al.* submitted, Tanio *et al.* 2005). The results of this study were consistent with those of previous studies. However, the effect of *Ppd-B1a* could not be determined, because cultivars with the *Ppd-B1a/Ppd-D1b* genotype were not found in the Japanese improved cultivars. Tanio and Kato (2007) analyzed 'Haruhikari' NILs with different *Ppd-1* genotypes and revealed that photoperiodic response was smallest in the *Ppd-B1a/Ppd-D1a* genotype followed by the *Ppd-B1a/Ppd-D1b*, *Ppd-B1b/Ppd-D1a*, and *Ppd-B1b/Ppd-D1b* genotypes. The result, summarized in Table 4, also showed that the *Ppd-B1a/Ppd-D1a* genotype headed 4.9–8.7 days earlier than the *Ppd-B1b/Ppd-D1a* genotype, indicating a significant effect of *Ppd-B1a* in the genetic background with *Ppd-D1a* and suggesting that early-maturity wheat breeding in Japan has been accelerated by the introduction of the *Ppd-B1a* allele.

Although the Bangladeshi cultivar 'Jessore' was considered the donor of the *Ppd-B1a* allele in extremely early cultivars in Japan (Tanio and Kato 2007, Yoshida *et al.* 1983), the *Ppd-1* genotype proved to be *Ppd-B1b/Ppd-D1a* (Fig. 4). Therefore, as summarized in Fig. 4, we concluded that the old Japanese cultivar 'Shiroboro 21' was the *Ppd-B1a* donor. *Ppd-B1a* probably had been introduced from 'Kinki 14' for the breeding of early-heading wheat at the Chugoku Agriculture Experimental Station during the 1940s–1950s. As shown in Table 1, one of the introduced cultivars, 'Tapdongmil', also carried *Ppd-B1a*. Although 'Tapdongmil' is a Korean cultivar, 'Chugoku 81', bred at the Chugoku Agriculture Experimental Station, was used as one of the cross parents (Sung *et al.* 1987). Therefore, *Ppd-B1a* in 'Tapdongmil' must be inherited from 'Chugoku 81'.

Using near-isogenic lines of 'Haruhikari' with different *Ppd-1* genotypes, Tanio and Kato (2007) showed that NILs carrying the photoperiod-insensitive allele *Ppd-B1a* started floral development and stem elongation earlier than the other NILs. Three cultivars confirmed to carry *Ppd-B1a* are successfully grown in the Chugoku or Kyushu regions of Japan, where the average daily minimum temperature from 1971 to 2000 was above freezing all year (Japan Meteorological Agency 2010). In contrast, in the north Kanto region, where the average daily minimum temperature was below freezing for 2–3 months, the cultivars suffered from late frost because of early stem elongation and poor adaptability (Inamura *et al.* 1958). Reflecting such a climatic difference, no cultivars carried *Ppd-B1a* in eastern Japan.

Taya (1993) reported that yield decreased with the advancement of heading time because of the decrease in spikelet number. The photoperiod-insensitive allele *Ppd-1* genes also reportedly shortened the duration of spikelet initiation (Gonzalez *et al.* 2005, Scarth *et al.* 1985, Tanio and Kato 2007). In the present study, heading date of cultivars carrying two genes, namely, *Ppd-B1a/Ppd-D1a*, were earlier than those of the other *Ppd-1* genotypes. In addition, heading date differences between two crop years, 2005/2006 (cold winter) and 2006/2007 (mild winter), were larger in the *Ppd-B1a/Ppd-D1a* genotype than in the other genotypes (Table 5). These results suggest that grain yield of early-heading cultivars carrying the two photoperiod-insensitive genes *Ppd-B1a/Ppd-D1a* will be lower, especially in warm-winter seasons. However, Fujita *et al.* (1995) and Seki *et al.* (2007) reported that the yearly fluctuation of heading time is smaller in the winter-type NILs of 'Abukumawase' carrying *Ppd-B1a/Ppd-D1a*. The fluctuation of heading time due to early sowing is also estimated to be smaller in winter type wheat (Fujita *et al.* 1995). Therefore, to breed early-heading cultivars adaptable to the Kanto region, *Ppd-B1a* and *Ppd-D1a* genes should be introduced with adequate varnalization requirement.

The previous genetic analysis indicated that spring type cultivars originated in the Tohoku-Kyushu region carry the vernalization response gene *Vrn-D1* (Gotho 1979, Iwaki *et*

al. 2000), and the present study showed that most of the cultivars in the Tohoku-Kyushu region carried the photoperiod-insensitive gene *Ppd-D1a*. These results indicate that most of the Japanese spring type cultivars, except those in the Hokkaido region, have the same set of genes for both the vernalization response and the photoperiod response. However, heading time varies between cultivars. Therefore, to refine heading time, further study is required to determine the genetic factors involved, including *Ppd-A1* (Nishida *et al.* submitted), the FT-like gene known as the photoperiod response gene in barley (Kikuchi *et al.* 2009), and other QTLs for photoperiod response and earliness *per se*.

Acknowledgements

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